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EMBRYOLOGY OF *JUBAEOPSIS CAFFRA* BECC.: 2. MEGASPORANGIUM, MEGASPOROGENESIS AND MEGAGAMETOGENESIS

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ABSTRACT

The ovary of *J. caffra* contains three uniovulate locules. These ovules, which are without a funicle, are intermediate between orthotropous and hemitropous.

The ovule is crassinucellate and bitegmic with procambium strands in the outer integument only. The inner integument, which becomes tanniniferous, forms an operculum.

Megasporogenesis results in the formation of either a linear or T-shaped tetrad of megaspores. Megagametogenesis results in the formation of a Polygonum type embryo sac with persistent synergids and ephemeral antipodal cells. A postament is present in the mature embryo sac.

UITTREKSEL

EMBRIOLOGIE VAN *JUBAEOPSIS CAFFRA* BECC.: 2. MEGASPORANGIUM, MEGASPOROGENESE EN MEGAGAMETOGENESE

Die vrugbeginsel van *J. caffra* is drie-lokulêr met 'n enkele saadknop in elke hok. Hierdie saadknoppe besit geen funikulus nie en is intermediêr tussen ortotroop en hemitroop.

Die saadknop is krassinuselleer met twee integumente. Prokambium stringe kom slegs in die buite-integument voor. Die binne-integument, wat op 'n latere stadium tannienvormend word, vorm 'n operkulum.

Megasporogenese lei tot die vorming van òf 'n lineêre òf 'n T-vormige tetrade van megaspore. Megagametogenese lei tot die vorming van 'n Polygonumtipe embriosak met blywende sinergiede en efemere antipodale selle. 'n Postament is aanwesig in die volwasse embriosak.

INTRODUCTION

Apart from a publication on the microsporogenesis and microgametogenesis of *J. caffra* (Robertson, 1976), no other data relating to embryological aspects of this unique species are available.

In the Palmae generally, there appears to be a wide variation with respect to ovule structure. Davies (1966) states that palm ovules are anatropous to hemi-anatropous and bitegmic while Moore and Uhl (1973) report that many are in fact tri-tegmic, i.e. with an aril.

Three types of megagametogenesis have been described for the Palmae. The Allium or bisporic type (Maheshwari, 1950) occurs in *Chamaedorea latifolia* (Jonsson 1879–80 cited by Davis, 1966), *Nipa fruticans* (Radermacher, 1925 cited

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by Maheshwari, 1950) and *Hyphaena indica* (Mahabale & Chennaveeraiah, 1957).

The second type is reported by Quisumbing & Juliano (1927) for *Cocos nucifera*, viz. the tetrasporic, eight-nucleate or Adoxa type (Maheshwari, 1950). These authors admit however that their findings conflict with Bauch (1911, cited by Quisumbing & Juliano, 1927) who claims to have observed degenerating megaspores during the late stages of sporogenesis and one is consequently left in some doubt as to which of the above two reports is correct.

The majority of palms appear to follow the third developmental pattern, viz. the monosporic, eight-nucleate or Polygonum type (Maheshwari, 1950) of embryo sac development (Swamy, 1942; Venkato Rao, 1955a, b; 1956a, b; 1958; Davis, 1966).

The actual structure of the embryo sac is not constant in the Palmae. In most cases a postament or podium is absent while in other species, e.g. *Areca catechu* (Venkato Rao, 1958) it is present. So, too, there is a variation in the role played by the antipodals. In *Hyphaene indica* (Mahabale & Chennaveeraiah, 1957) the antipodal nuclei remain naked, in *Areca catechu* (Venkato Rao, 1958) they are persistent and aggressive while in most other studied species they are ephemeral (Davis, 1966).

MATERIAL AND METHODS

Initially female flowers were obtained from four entire inflorescences which were cut down at various stages of development. However, it soon became apparent that all the female flowers on a single inflorescence were more or less at the same stage of development. Consequently, in order to avoid the destruction of too many inflorescences, it was necessary to leave the inflorescence attached to the tree and simply split the woody covering bract longitudinally open and collect the flowers at weekly intervals.

These were fixed in either Craf II or F.A.A. (Sass, 1958), dehydrated in an ethyl alcohol/tertiary butyl alcohol (TBA) series, embedded in paraffin wax (55°C) and sectioned at 10 μm on a rotary microtome as prescribed by Brooks, Bradley and Anderson (1950). The sections were stained in safranin/fast green (Holtzhausen, 1972).

RESULTS AND DISCUSSION

Megasporangium

The ovary of *J. caffra* is comprised of three carpels whose loculi extend upwards in the form of a 3-radiate canal (Fig. 1) to join the stylar canal.

Placentation of the ovules is free-central (Fig. 1). The ovule is without a funicle and arises directly from the placenta (Figs 1, 2). Ovules of the genus *Bactris* are also without funicles (Venkato Rao, 1958).

In a longitudinal section through the ovary, the ovule is curved downwards by approximately 45° (Figs 1, 2, 5A) and consequently it is neither truly orthotropous nor fully hemitropous. This is in contrast to the general condition found in the Palmae as a whole where the ovule is basically anatropous (Eames, 1961).

The ovule is bitegmic and the two integuments are fused together up to a point approximately midway between the chalaza and the micropylar end of the nucellus (Fig. 2). Initially the outer integument does not enclose the inner one (Fig. 3) suggesting that differentiation of the inner integument is initiated before that of the outer. By the time that the megaspore mother cell has differentiated though, the outer integument fully envelops the inner one as well as the nucellus and both integuments form the micropyle. The exostome and endostome are in line with each other and consequently the micropyle is straight, and points slightly downwards (Fig. 5A).

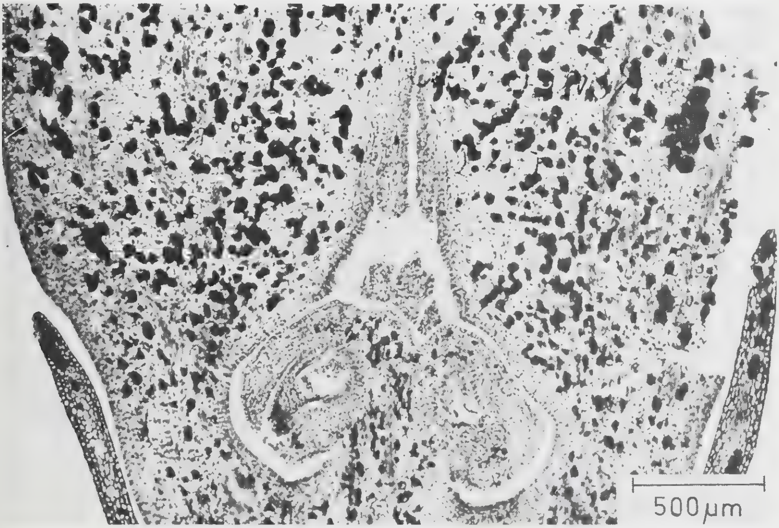
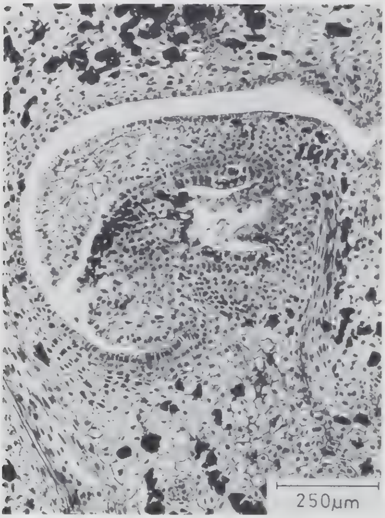


FIG. 1.
Longitudinal section through a female flower of *J. caffra* showing the freecentral placentation of the ovules.

The outer integument is between five and seven cell layers thick and dominates the ovule by its massive structure. During megasporogenesis and the early stages of megagametogenesis the cells of the outer integument are fairly homogeneous, but by the time the embryo sac reaches maturity, the epidermis cells of the distal portion of this integument become anticlinally elongated (Fig. 2). At this stage too, the cells constituting the central tissues of the outer integument become disorganized.

The inner integument is a much more delicate structure and along the sides of the nucellus it is usually only two cell layers thick. Occasionally it may be three cell layers thick at this point (Fig. 6C). At the micropyle, the inner integument widens to form an operculum (Figs 6B, C). The cells of the operculum start to accumulate tannin from a relatively early stage and by the time the embryo sac reaches maturity, many of the operculum cells are tanniniferous (Figs 6B–F, 7).



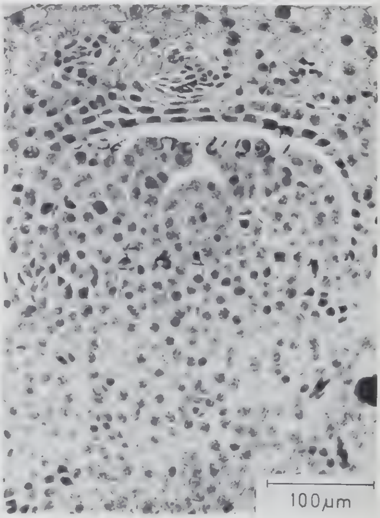


FIG. 2.
Longitudinal section through the ovule during the late stages of megagametogenesis. No funicle is present.

FIG. 3.
Transverse section through a portion of the ovary showing a young ovule, longitudinally sectioned, during differentiation of the integuments.

During megasporogenesis the cells of the inner integument undergo differentiation. The cells of the inner epidermis of this integument, i.e. the integumentary cells lying directly adjacent to the nucellus elongate radially to form an integumentary tapetum or endothelium (Figs 5C, 6A–D). They remain uninucleate and divide anticlinally to keep pace with the increasing volume of the developing embryo sac.

In *J. caffra* the nucellus at the chalazal end of the embryo sac remains intact until after fertilization, but the nucellar tissue in the micropylar half of the ovule degenerates from a relatively early stage during megagametogenesis (Figs 6C, D) and contrary to the general statement made by McLean & Ivimey-Cook (1964) it is felt that although the endothelium might well be involved in the nutrition of the embryo sac, the protective and restraining function of this layer of cells must not be overlooked.

With regard to the nucellus of the ovule, two terms are in general current use, viz. crassinucellate and tenuinucellate. Unfortunately these two terms have been rather loosely used (Davis, 1966) and various authors have defined them differently. Maheshwari (1950) and Davis (1966) use the presence or absence of one or more parietal cell layers between the megaspore mother cell and the nucellus epidermis as the criterion for distinguishing between crassinucellate and tenuinucellate ovules. Eames (1961) also makes use of the parietal tissue in his definitions, but considers the actual structure of the nucellus as a whole, i.e. whether it is massive, or small and delicate, as a more important criterion. McLean & Ivimey-Cook (1964) on the other hand disregard the parietal tissue entirely and use only the size (massive or delicate) and the longevity (relatively persistent or ephemeral) to differentiate between the two types.

The hypodermal archesporial cell of *J. caffra* (Fig. 4) divides periclinally to form a single parietal layer and the megaspore mother cell (Fig. 5A). Thus according to the definitions of Maheshwari (1950) and Davis (1966) the ovule of *J. caffra* is crassinucellate. Figs 5A, 5C, 6B however, show that the nucellus is by no means massive but rather, in the words of Eames (1961) ''small and delicate''. The nucellus is also not very persistent (except for the postament) but rather ephemeral. Consequently the ovule could be considered tenuinucellate if one was to employ the definitions of either Eames (1961) or McLean & Ivimey-Cook (1964).

The definitions of Maheshwari (1950) and Davis (1966) are perhaps more standard than those of Eames (1961) and McLean & Ivimey-Cook (1964) and consequently the ovule of *J. caffra* will be classified as crassinucellate.

It is felt that the term crassinucellate in general is too wide and that a more specific terminology should be devised for more precise descriptions of this type of nucellus. Maheshwari (1950), for example, describes two types of tenuinucellate ovules, depending on the position of the point of origin of the integuments.

The nucellus closest to the micropyle starts to degenerate at an early stage in the development of the female gametophyte (Figs 6C, D) and by the time the embryo sac reaches maturity only a single, disorganized layer of nucellus cells lies between the embryo sac and integument (Fig. 7). The nucellus at the chalaza however is persistent and in a longitudinal section through the ovule it can be observed as two ''horns'' stretching from the chalaza down towards the micropyle (Fig. 7). A similar nucellar structure, i.e. a postament occurs in a number of other palm species, e.g. *Caryota mitis, Chrysalidocarpus lutescens, Actinophloeus macarthurii, Howea belmoreana, Areca triandra* and *A. concinna* (Venkato Rao, 1958). The postament does not persist very long after fertilization and cannot be observed after embryo development has started.

Vascular tissue from the placenta to the ovule mostly terminates in the chalazal region. A small amount of vascular tissue continues into the outer integument, but it is relatively poorly differentiated and occurs mainly in the form of procambium strands.

Archesporium

Very early in the ontogeny of the ovule, before the primordia of the integuments are visible, a single hypodermal cell in the apex of the nucellus becomes conspicuously larger with denser cytoplasm than the surrounding nucellus cells (Fig. 4). This cell constitutes the archesporium of *J. caffra*.

As development of the ovule progresses, the archesporial cell divides periclinally to produce the primary parietal cell and the primary sporogenous cell. No further periclinal divisions occur in the former cell. It does however divide anticlinally to keep pace with the increase in volume of the nucellus. The single megaspore mother cell, which normally differentiates directly from the primary

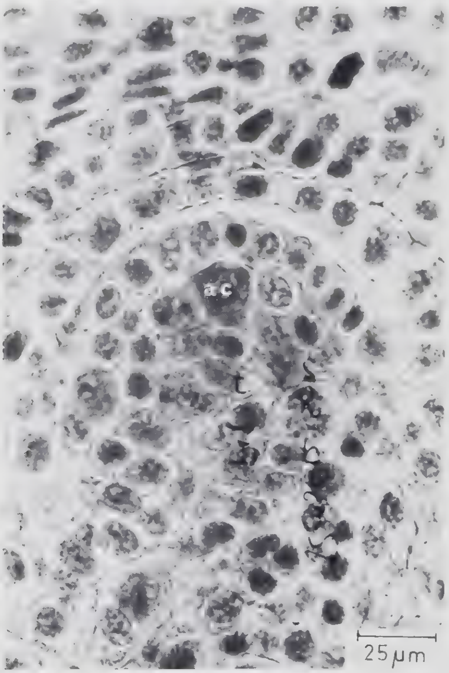


FIG. 4.
Longitudinal section through nucellus primordium in which the archesporial cell (ac) has differentiated. No integumentary development has as yet been initiated.

sporogenous cell without any intervening divisions, is thus separated from the nucellus epidermis by a single cell layer of parietal tissue.

Abnormal ovules occasionally occur in which more than one megaspore mother cell are present.

The archesporial development of *J. caffra* thus conforms to Type III as described by Schnarf (cited by McLean & Ivimey-Cook, 1964) for angiosperms generally.

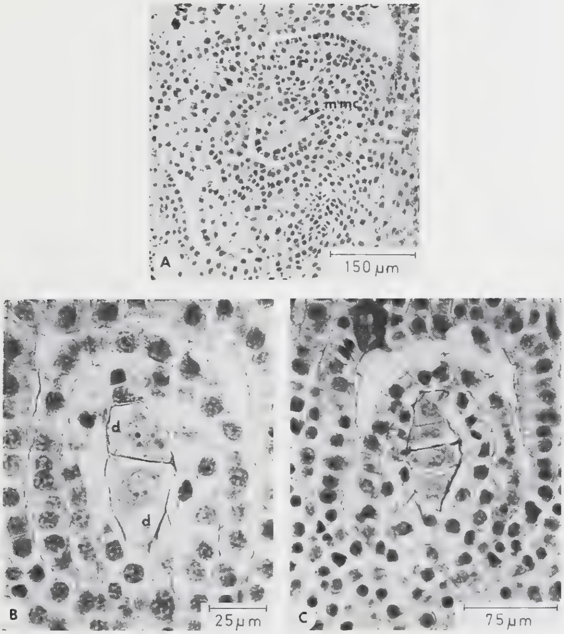
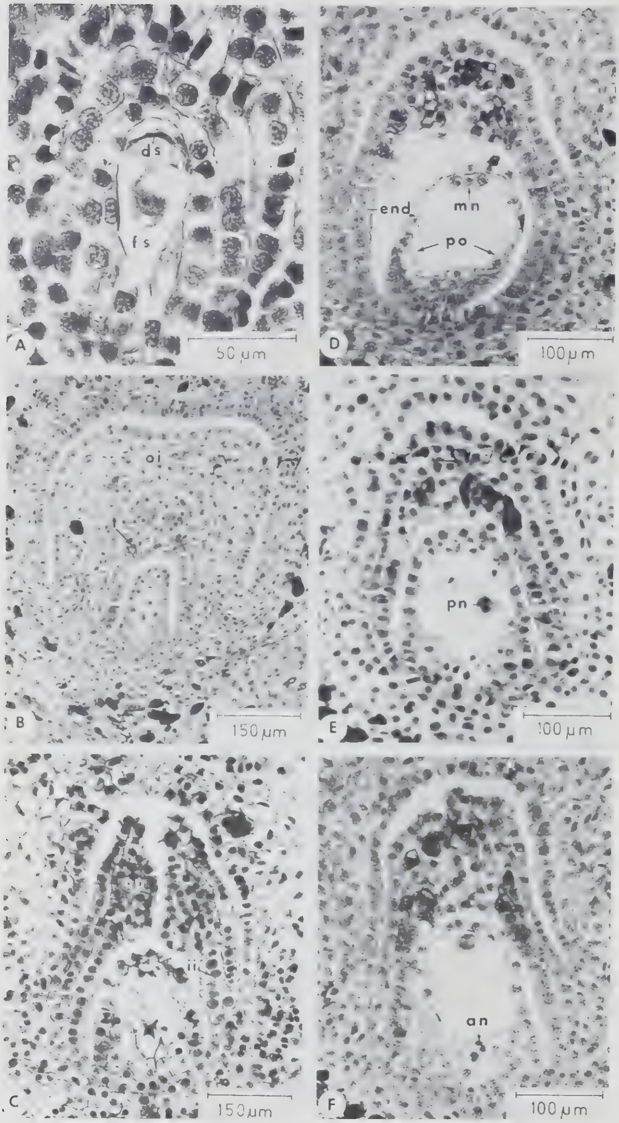


FIG. 5.
Megasporogenesis in *J. caffra*. A – Megaspore mother cell (mmc). B – Megaspore dyad cells (d). C – Linear tetrad of megaspores.

Megasporogenesis

The mature megaspore mother cell is relatively large and typically it is elongated parallel to the long axis of the nucellus (Fig. 5A). This mother cell contains a large nucleus with a conspicuous nucleolus.

Megasporogenesis is successive, i.e. cell walls are laid down after each of the two division during meiosis. The megaspore mother cell undergoes meiosis and as always, the first division is transverse to the long axis of the nucellus to form two dyad cells (Fig. 5B). Each of these two cells divides again to form the megaspore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tetrad. In all cases the chalazal dyad cell divides transversely. The micropylar dyad cell can however divide either transversely to form a linear tetrad (Fig. 5C) or longitudinally to form a T-shaped tetrad. The T-shaped tetrad appears to be more common than the linear one. In the T-shaped tetrad, the division of the micropylar dyad cell occurs in such a plane that only three megaspores are visible in a single transverse section through the ovary while the fourth megaspore lies either directly above or below the micropylar spore (still in transverse section).

The chalazal cell of the tetrad always functions and gives rise to the embryo sac while the three micropylar megaspores degenerate and become crushed by the expanding embryo sac (Fig. 6A).

Megagametogenesis

The development of the female gametophyte is initiated by the enlargement of the chalazal megaspore only (Fig. 6A), and the female gametophyte is therefore monosporic.

Enlargement of the functional megaspore is followed by three mitotic divisions, resulting in the formation of a 2-, 4- and eventually 8-nucleate embryo sac (Figs 6B–F).

Of these eight nuclei, three remain in the micropylar region of the embryo sac to form the egg apparatus (Fig. 6D); one nucleus from each pole migrates to the centre of the embryo sac where they fuse to form the secondary nucleus (Fig. 6E) and three remain in the chalazal end to form the antipodals (Fig. 6F).

Based on the structure of the mature embryo sac, the female gametophyte of *J. caffra* thus conforms to the Polygonum type (Maheshwari, 1950).

The two synergids are definite cells each with a large nucleus and nucleolus, very dense and dark staining cytoplasm and a series of small vacuoles arranged along the periphery of each cell, especially along the cell wall furthest from the micropyle, i.e. towards the inside of the embryo sac (Fig. 7). No hooks are formed and the synergids are attached to the embryo sac wall by a broad base. The free sides of these two cells are slightly rounded. No filiform apparatus is visible.

One of the synergids disappears just prior to fertilization while the other starts to degenerate shortly after fertilization. Its remains can however be observed until the pro-embryo is approximately eight- to sixteen-celled.

FIG. 6.

Megagametogenesis of *J. caffra*. A – Functional chalazal megaspore plus degenerating spores. B – Two-nucleate embryo sac. C – Four-nucleate stage of female gametophyte. Only three nuclei are visible in this figure. Note the operculum of the inner integument. D – Three micropylar nuclei which will form the egg apparatus. E – Fusion of the two polar nuclei during formation of the secondary nucleus. F – Three antipodal nuclei. Also visible are two micropylar nuclei.

(an—antipodals; ds—degenerating spores; end—endothelium; fs—functional megaspore; ii—inner integument; mn—micropylar nuclei; oi—outer integument; op—operculum; pn—polar nuclei; po—nucellar postament; t—tanniniferous cells).

The egg cell is somewhat smaller than the synergids and is situated slightly laterally to them with the result that all three cells of the egg apparatus can very rarely be observed in a single longitudinal section.

Three antipodal cells are present (Fig. 6F). These cells are small and relatively ephemeral, disappearing shortly after the formation of the secondary nucleus. By the time that the synergids are fully differentiated (Fig. 7) no trace of the antipodals is left.

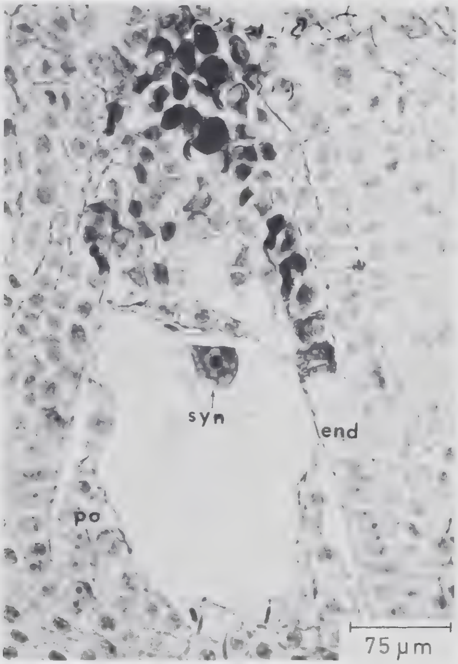

Fig. 7.
A fully differentiated synergid.
(end—endothelium; po—podium; syn—synergid).

Initially, i.e. up to the 2-nucleate stage, the embryo sac is a long narrow structure (Figs 6A, B). From this stage on however, the embryo sac enlarges very rapidly in both a longitudinal and transverse direction with the result that the nucellus, particularly in the vicinity of the micropyle, degenerates. The mature embryo sac is approximately 170 μm long and 120 μm wide.

Conclusions

In general the megasporogenesis and megagametogenesis of *J. caffra* is in accordance with that of the Cocoid palms studies by Venkato Rao (1958), viz.

Bactris major and *Arecastrum romanzoffianum* and a Polygonum-type embryo sac develops from the functional chalazal megaspore of the megaspore tetrad.

It is strange though that the embryology of *Cocos nucifera* and *J. caffra* should be so vastly different. These two species are very similar in many aspects, yet Quisumbing & Juliano (1927) report that a bisporic, Allium type of female gametophyte is found in *Cocos*. They differ further in that the archesporial cell of *J. caffra*, which becomes visible prior to differentiation of the integuments, divides to give rise to a parietal cell and a sporogenous cell, the latter of which differentiates into the megaspore mother cell; while in *Cocos*, the archesporial cell differentiates directly into the megaspore mother cell, or as Quisumbing & Juliano put it ". . . becomes the megaspore mother cell". Furthermore this cell (in *Cocos*) is not visible until both the integuments are fully differentiated (Quisumbing & Juliano, 1927).

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REFERENCES

BROOKS, R. M., BRADLEY, Muriel V. and ANDERSON, Thelda I., 1950 *Plant microtechnique manual*. Davis: University of California.

DAVIS, Gwenda L., 1966. *Systematic embryology of the Angiosperms*. New York: John Wiley & Sons, Inc.

EAMES, A. J., 1961. *Morphology of the Angiosperms*. New York: McGraw-Hill Book Company.

HOLTZHAUSEN, L. C., 1972. *'n Morfo-genetiese en fenologiese studie van die blom en vrug van* Citrus sinensis *(L.) Osbeck., Cultivar Valencia*. University of Pretoria: D.Sc. (Agric.) thesis.

MAHABALE, T. S. and CHENNAVEERAIAH, M. S., 1957. Studies on *Hyphaene indica* Becc. I. Morphology. *Phytomorphology* **7:** 184–94.

MAHESHWARI, P., 1950. *An introduction to the embryology of Angiosperms*. New York: McGraw-Hill Book Company.

MCLEAN, R. C. and IVIMEY-COOK, W. R., 1964. *Textbook of Theoretical Botany*. Vol. 2. London: Longmans.

MOORE, H. E., JR., and UHL, Natalie, 1973. The monocotyledons: Their evolution and comparative biology VI. Palms and the origin and evolution of monocotyledons. *Q. Rev. Biol.* **48:** 414–36.

QUISUMBING, E. and JULIANO, J. B., 1927. Development of the ovule and embryo sac of *Cocos nucifera*. *Bot. Gaz.* **84:** 279–93.

ROBERTSON, B. L., 1976. Embryology of *Jubaeopsis caffra* Becc. 1. Microsporangium, microsporogenesis and microgametogenesis. *Jl S. Afr. Bot.* **42** (2): 97–108.

SASS, J. E., 1958. *Botanical microtechnique*. Iowa: Iowa State University Press.

SWAMY, B. G. L., 1942. Embryological studies in Palmae. A preliminary note on the megasporogenesis in *Areca catechu*. *Curr. Sci.* **11:** 109.

VENKATA RAO, C., 1955a. Embryological studies in Palmae. I. *Proc. 42nd Indian Sci. Congr.* **3:** 230–31.

VENKATA RAO, C., 1955b. Embryological studies in Palmae II. *Proc. 42nd Indian Sci. Congr.* **3:** 231.

VENKATA RAO, C., 1956a. Embryological studies in Palmae IV. *Proc. 43rd Indian Sci. Congr.* **3:** 225–26.

VENKATA RAO, C., 1956b. Embryological studies in Palmae V. *Proc. 43rd Indian Sci. Congr.* **3:** 226.

VENKATA RAO, C., 1958. Contributions to the embryology of Palmae. *J. Indian bot. Soc.* **38:** 46–75.